DOI: 10.1534/genetics.107.078857

Genetic Analysis of Dauer Formation in *Caenorhabditis briggsae*

Takao Inoue,* Michael Ailion,[†,2] Shirley Poon,* Hannah K. Kim,*[,3] James H. Thomas[†] and Paul W. Sternberg*[,1]

**HHMI and Division of Biology, California Institute of Technology, Pasadena, California 91125 and* [†]*Department of Genome Sciences, University of Washington, Seattle, Washington 98195*

Manuscript received July 13, 2007
Accepted for publication July 18, 2007

ABSTRACT

Molecular changes that underlie evolutionary changes in behavior and physiology are not well understood. Dauer formation in *Caenorhabditis elegans* is a temperature-sensitive process controlled through a network of signaling pathways associated with sensory neurons and is potentially an excellent system in which to investigate molecular changes in neuronal function during evolution. To begin to investigate the evolution of dauer formation in the genus Caenorhabditis at the molecular level, we isolated dauer-formation mutations in *C. briggsae*, a species closely related to the model organism *C. elegans*. We identified mutations in orthologs of *C. elegans* genes *daf-2* (insulin receptor), *daf-3* (Smad), and *daf-4* (TGF-β type 2 receptor), as well as genes required for formation of sensory cilia. Phenotypic analyses revealed that functions of these genes are conserved between *C. elegans* and *C. briggsae*. Analysis of *C. briggsae* mutations also revealed a significant difference between the two species in their responses to high temperatures (>26°). *C. elegans* is strongly induced to form dauers at temperatures above 26°, near the upper limit for growth of *C. elegans*. In contrast, *C. briggsae*, which is capable of growth at higher temperatures than *C. elegans*, lacks this response.

CAENORHABDITIS briggsae is a nematode closely related to the model organism *C. elegans* (reviewed in Gupta *et al.* 2007). A range of molecular, genetic, and genomic resources makes this species an ideal target of comparative studies with *C. elegans*. The genome of *C. briggsae* has been sequenced and is annotated in detail (Stein *et al.* 2003). Since *C. briggsae* propagates as self-fertilizing hermaphrodites like *C. elegans*, genetic analysis of *C. briggsae* is facile, and a number of labs have produced genetic tools [*e.g.*, morphological markers or single nucleotide polymorphism markers (Hillier *et al.* 2007)] that are useful in genetic mapping and strain construction (Gupta *et al.* 2007). Molecular manipulations including transformation (Kennedy *et al.* 1993; Kirouac and Sternberg 2003) and RNAi (Rudel and Kimble 2001; Nayak *et al.* 2005) are possible in *C. briggsae*. Like *C. elegans*, *C. briggsae* has nearly invariant cell lineages that produce identical sets of cells in every animal. The complement of cells is also conserved between *C. elegans* and *C. briggsae*, providing an opportunity to compare functions of individual cells (Delattre and Felix 2001).

[1]*Corresponding author:* HHMI and Division of Biology, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA. E-mail: pws@caltech.edu

[2]*Present address:* Department of Biology, University of Utah, Salt Lake City, UT 84112.

[3]*Present address:* Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853.

Despite their similarities, *C. briggsae* and *C. elegans* are distinct species with overlapping geographic distributions and probably occupy different ecological niches (Cutter *et al.* 2006). One known difference of possible ecological significance is in their temperature responses. All isolates of *C. elegans* grow at temperatures from ~10° to 27° (M. Ailion and J. H. Thomas, unpublished data). In contrast, *C. briggsae* can be cultured at temperatures over 29°. This difference in temperature preferences suggested that dauer formation response might also differ between the two species. As with *C. elegans* (Cassada and Russell 1975), *C. briggsae* responds to unfavorable environmental conditions (especially high population density, absence of food bacteria, and high temperature; Golden and Riddle 1984b) by entering the dauer stage, which is a developmentally arrested third larval stage (Fodor *et al.* 1983). This response is mediated in part by dauer pheromones, secreted molecules that induce dauer formation in *C. elegans* and *C. briggsae* (Golden and Riddle 1982; Jeong *et al.* 2005; Butcher *et al.* 2007). Dauers exhibit specific morphological, physiological, and behavioral characteristics that enable them to survive harsh conditions and may aid in dispersal (Cassada and Russell 1975). When a favorable environment is encountered, dauers reenter the normal life cycle and go on to become normal adults. In *C. elegans*, dauer formation in the 15° to 25° range shows broad temperature sensitivity with higher temperatures promoting entry into the dauer stage (Golden and Riddle

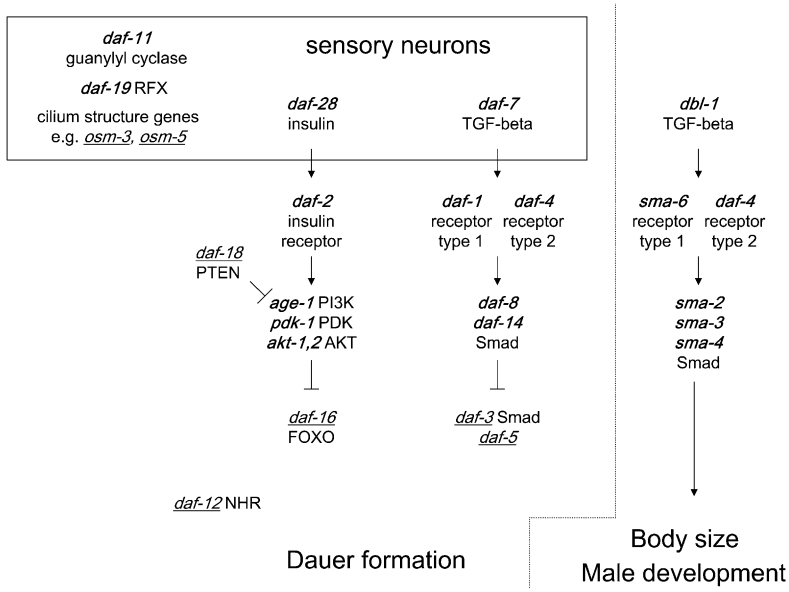

FIGURE 1.—Genetic pathways regulating dauer formation in *C. elegans*. This figure shows a subset of genes that mutate to affect dauer formation. Genes that cause constitutive dauer formation when mutated are shown in boldface and genes that cause defective dauer formation are underlined. Genes that function in neurons are shown in the box at the top. Genes in each pathway (TGF-β, insulin) usually demonstrate clear epistasis relationships. Interactions between mutations that affect different pathways are complex. The paralogous pathway regulating body size and male tail development is shown to the right.

1984a,b). Furthermore, at temperatures near the upper limit of growth and reproduction (>26°, typically assayed at 27°), there is exceptionally strong induction of dauer formation (AILION and THOMAS 2000). Several lines of evidence indicate that this high-temperature response is qualitatively distinct from temperature sensitivity in the 15° to 25° range. For example, some genes that promote dauer formation at lower temperatures inhibit dauer formation at higher temperatures (AILION and THOMAS 2000). The observation that *C. briggsae* is capable of growth at higher temperatures (>29°) raised an interesting question: Does *C. briggsae* exhibit a similar response, and if it does, at what temperature?

To begin to investigate evolutionary changes in mechanisms that regulate dauer formation, we isolated and characterized mutations in *C. briggsae* that affect dauer formation. Analogous mutations in *C. elegans* have been studied for many years (Figure 1) (RIDDLE *et al.* 1981; THOMAS 1993). Together, these mutations enable us to compare the functions of dauer-regulating genes in the two species. From several screens we identified mutants that fail to form dauers normally when exposed to dauer-inducing conditions (dauer-formation defective or Daf-d mutants) and mutants that form dauers even in dauer noninducing conditions (dauer-formation constitutive or Daf-c mutants). Phenotypic analyses and mapping suggested molecular identities for some mutations, and sequencing confirmed three to be mutations in orthologs of *daf-2* (insulin receptor), *daf-3* (Smad), and *daf-4* (TGF-β type 2 receptor). Phenotypes and genetic interactions demonstrate that functions of these genes are conserved between *C. elegans* and *C. briggsae*. Furthermore, using these mutations, we tested high-temperature dauer-formation responses in *C. briggsae*. Although dauer formation in *C. briggsae* is broadly temperature sensitive, we found no evidence of dauer hyperinduction at high temperatures in *C. briggsae*.

MATERIALS AND METHODS

Genetics and nomenclature: We used the *C. briggsae* isolate AF16 (available from Caenorhabditis Genetics Center, University of Minnesota) as the wild-type strain. This strain is thought to exhibit wild-type dauer formation behavior (FODOR *et al.* 1983). All strains described in this article are in this background. Mutations used for mapping are LGI *sma(sy5330)*, LGII *cby-15(sy5148)*, *Cb-unc-4(sy5341)*, LGIII *cby-4(sy5016)*, LGIV *mip-1(s1270)*, LGV *unc(sa997)* LGX *cby-3(sy5039)*, *rot-1(sy5001)*, and *unc(sa988)*. *cby-4* and *mip-1* are thought to be orthologs to *dpy-1* III and *unc-22* IV respectively.

We use the guideline for nomenclature of non-*C. elegans* strains extended from the published guideline for *C. elegans* (HORVITZ *et al.* 1979; HODGKIN 1995). Briefly, strain names and mutation names (allele numbers) follow the same format as *C. elegans*. There is no overlap with *C. elegans* strain and allele designations. Where orthology to a *C. elegans* gene is known, the gene name reflects the orthology, *e.g.*, *Cb-daf-4* is the *C. briggsae* ortholog of *C. elegans daf-4*. Different gene/phenotype names are used for *C. briggsae* genes whose orthology has not been determined. Corresponding names are (*C. briggsae*/*C. elegans*): Cby (chubby)/Dpy (dumpy), short and fat; Rot (rotator)/Rol (roller), spirally twisted animals; Mip (movement impaired)/Unc (uncoordinated), abnormal movement. Orthology and sequence information were obtained from WormBase (data freeze WS140). *Cb-daf-2*, *Cb-daf-3*, and *Cb-daf-4* are CBG15732, CBG08108, and CBG08963.

Isolation and characterization of *C. briggsae* Daf-d mutations: Ethylmethanesulfonate (EMS) mutagenesis was carried out as described for *C. elegans*, except in some cases animals were incubated in EMS solution for three hours instead of four (BRENNER 1974). Mutagenized worms were allowed to self for two generations. F_2 animals were picked one per plate. These plates were allowed to grow until starvation, at which time dauer formation was tested by flooding the plate with 1% SDS (sodium dodecyl sulfate) solution in water. All larvae except dauers are killed by 1% SDS in ~15 min (CASSADA and RUSSELL 1975). Plates on which all animals were killed by SDS were identified as candidate Daf-d mutations. To allow recovery of such Daf-d mutations, a replicate of each plate was made by transferring a small piece of agar with worms to a new plate (chunking) prior to the SDS test. On further retests, we found that some mutations caused a complete block of dauer formation, whereas

others formed some dauers under starvation conditions. We focused our analysis primarily on mutations that completely blocked dauer formation.

To test for the morphology of dauers, animals were mounted for Nomarski observation (Sulston and Horvitz 1977) and scored for dauer alae and modified pharynx. To test for a dye-filling defect, animals were stained with the DiI solution as described (Prasad *et al.* 1998).

To isolate phenotypic suppressors of the Daf-c mutation *Cb-daf-4(sa973)*, we mutagenized with EMS a strain carrying *Cb-daf-4(sa973)*. The F_1 population was allowed to self at the permissive temperature (20°), and then the plates were shifted to 28°. Non-dauer F_2's were picked, selfed to establish a line, and retested for dauer formation at 28°. From a screen of 4200 mutagenized genomes, we recovered eight independent mutations, of which one (*sy5321*) was analyzed in detail. *sy5321* suppresses the Daf-c phenotype but not the Sma phenotype at the restrictive temperature for *Cb-daf-4(sa973)*. *Cb-daf-4(sa973); sy5321* animals were crossed to wild-type males, and progeny were allowed to self. Twenty-four randomly selected F_2 animals were picked individually and allowed to self at 28°. Plates that had no dauers or Sma animals were allowed to grow until starvation and tested for the presence of dauers. Lines that segregated no Sma animals and did not form dauers were retained as an outcrossed *sy5321* strain.

To map Daf-d mutations to the X chromosome, we crossed AF16 males to *daf-d/ daf-d* hermaphrodites, and male progeny were crossed to an X-linked marker strain, most often PS9353 *cby-3(sy5039) rot-1(sy5001)* X. Cross-progeny hermaphrodites were allowed to self, and a number of phenotypically wild-type F_2 progeny were picked individually to ~20 plates. In the next generation, we selected plates that segregated no Cby Rots. These were allowed to starve, and examined for the presence of dauers. If the mutation was X-linked, very few or no dauers were observed. If the mutation was not X-linked, a majority of the plates segregated a large number of dauers.

The ability of *daf-d* mutations to suppress *Cb-daf-4(sa973)* was tested as follows. *daf-4/+* males were crossed to *daf-d/daf-d* homozygotes. Progeny were picked to individual plates and allowed to self at 28°, where *daf-4/+* cross-progeny segregated dauers. If the *daf-d* mutation suppressed the Daf-c phenotype of *daf-4*, we would observe Sma non-Daf-c animals segregating on the plate (*daf-4/daf-4; daf-d/daf-d* animals: 1/16 of the total population). Such animals were picked individually and allowed to self at 28° to confirm the presence of *daf-4* and to confirm suppression. This test assumes that the *daf-d* mutation suppresses the Daf-c but not the Sma phenotype. No mutation in either species is known to suppress both phenotypes of *daf-4*.

Isolation and characterization of *C. briggsae* Daf-c mutations: Screens for Daf-c mutants in the wild-type background were carried out as described (Riddle 1977). Screens for revertants of *Cb-daf-3* were carried out as follows. A *Cb-daf-3(sy5417)* strain was EMS mutagenized and allowed to self at 25° for two or more generations, until the plates were starved. Animals were washed off from starved plates and were treated with 1% SDS solution for 15 min to kill non-dauers. The surviving population was washed again in water and transferred to fresh plates seeded with *Escherichia coli*. After recovery, these animals were allowed to self for multiple generations until starved and reselected using 1% SDS. Animals recovered from the second round of selection were picked on to fresh plates, and dauer formation was tested visually at different temperatures. Because the selection was carried out in bulk, and not every recovered animal was picked at the end, the number of genomes screened is unknown.

To map *Cb-daf-2(sy5445)*, we allowed *sy5445/cby-4* heterozygotes to self at 28°. Dauer progeny (*sy5445* homozygotes) were picked and allowed to recover at 20°. Recovered dauers were selfed at 20°, and segregation of Cby (Dpy) animals was scored. None of seven *sy5445* homozygotes segregated *cby-4*, indicating linkage to chromosome 3. To test whether *Cb-daf-2(sy5445)* extends lifespan, we picked mutant and wild-type animals in the L4 stage to plates seeded with OP50. Animals were transferred to fresh plates as necessary, for example because of overcrowding by progeny. Plates were checked every day for deaths, scored by the lack of movement. Animals that died because of unnatural causes, *e.g.*, desiccation on the side of the plate, were not counted.

TABLE 1

Primers used in this study

Primer	Sequence	Gene
CB39	GCACCTACAAACATGACTAC	*Cb-daf-3*
CB40	CTGTTGAGATCGTATGTAAC	*Cb-daf-3*
CB41	CATCCATGAACTTGTATGCTC	*Cb-daf-3*
CB8	ACCGACTGCCGACCCTGTTG	*Cb-daf-4*
CB9	CCCTCTACCGTATATTTCGG	*Cb-daf-4*
CB11	TAAGGTAATCATGAACCTATC	*Cb-daf-4*
CB15	GAAAATCGAGAAAACGGGAAC	*Cb-daf-4*
CB101	TCGGTGACGGAGACTGGAAA AGTATGCCC	*Cb-daf-2*
CB102	GGGTGTTCGGGCGGATACGG TCTTTTGCC	*Cb-daf-2*
CB103	TTCGAGGCGATGCGTTTGGCC CATGCGCC	*Cb-daf-2*
CB104	TCATCCTTCTACCGCCCTTCC TCTCCTCC	*Cb-daf-2*

Sequencing of *Cb-daf-2(sy5445)*, *Cb-daf-3(sy5417)*, and *Cb-daf-4(sa973)*: The MH2 (C-terminal) domain of *Cb-daf-3* was amplified from animals bearing the *sy5417* mutation using primers CB39 and CB40 (Table 1). The amplified fragment was sequenced directly using the primer CB41. The mutation destroys an SfaNI restriction site present in the wild-type genomic sequence. Therefore to confirm the wild-type and mutant sequences, we amplified genomic sequences from *sy5417* and wild-type animals using CB39 and CB40 and digested the fragments with SfaNI. The fragment from the wild type but not the mutant was digested by SfaNI at this site, confirming the change. The screen from which *daf-3(sy5417)* was isolated also produced two additional X-linked Daf-d mutant lines, to which were assigned allele numbers *sy5418* and *sy5419*. When these strains were tested for the presence of the SfaNI site, they were found to lack the SfaNI site. *sy5418* and *sy5419* also failed to complement *sy5417*. Thus *Cb-daf-3(sy5417)*, *sy5418*, and *sy5419* are the same mutation. Since *sy5417*, *sy5418*, and *sy5419* are derived from different mutagenized P_0 animals in the screen, this probably indicates that this was a spontaneous mutation present in the parent population at a small frequency. Another X-linked Daf-d mutant, *sy5315* does not have the mutation at the SfaNI site and complements *sy5417*.

We sequenced the kinase domain of *Cb-daf-4* from the mutant by amplifying the genomic sequence with primers CB8 and CB9 and sequencing the fragment directly with primers CB11 and CB15.

Although most *daf-2* mutations in *C. elegans* reside in two large exons, the gene structure of *Cb-daf-2* is different, and the homologous regions are spread out over a larger region of the genome (WormBase data freeze WS160). Therefore, we RT-PCR amplified these regions from mRNAs of mixed stage AF16 and *sy5445* strains using primer pairs CB101/CB102 and CB103/CB104, and the PCR products were sequenced directly

TABLE 2

Daf-d mutants of *C. briggsae*

Mutation	Gene	Class	Linkage	Dyf	Other
sy5311		Cilium	III	Dyf(−)	
sy5314		Cilium		Dyf(−)	
sy5420		Cilium		Dyf(−)	
sy5421		Cilium		Dyf(−)	
sy5313		*daf-13*-like	Autosomal		
sy5416		*daf-13*-like	Autosomal		
sy5429		*daf-13*-like			
sy5417	*Cb-daf-3*		X	Dyf(+)	Suppresses *Cb-daf-4(sa973)*
sy5315			X	Dyf(+)	Makes partial dauers
sy5312			Autosomal	Dyf(+)	
sy5431			Autosomal	Dyf(+)	
sy5443			Autosomal	Dyf(+)	
sy5444			Autosomal	Dyf(+)	
sy5442			Autosomal		
sa934		Cilium	Autosomal	Dyf(−)	
sy5321			X	Dyf(+)	*Cb-daf-4(sa973)* suppressor

sy5321 was isolated as a suppressor of *Cb-daf-4(sa973)*. *sa934* was isolated in a separate screen for Dyf mutants.

using the same primers. The *sy5445* mutation was confirmed in the sequence of both strands. The same mutation is absent in our wild-type cDNA sequence and the published genomic sequence (WormBase data freeze WS160).

Rescue of *Cb-daf-4* and generation of integrated lines: To test rescue of the *Cb-daf-4(sa973)* mutant, we coinjected a *Ce-daf-4* clone (Estevez *et al.* 1993) along with *Ce-myo-2::gfp* as a coinjection marker (A. Fire, personal communication). Two stable transgenic lines were generated by following *Ce-myo-2::gfp*. Transgenic animals were grown at restrictive temperatures (25° and 27°) to test for rescue. The integration of *saEx[myo-2::gfp, Ce-daf-4(+)]* was done as described, using the rescue of the *Cb-daf-4(sa973)* Sma phenotype as the marker (Way and Chalfie 1989). Integrants were mapped using chromosomal markers *sma(sy5330)* I, *cby-15* II, *cby-4* III, and *mip-1* IV.

RESULTS

Isolation and characterization of dauer formation defective mutants in *C. briggsae*: We carried out a screen for dauer-formation defective mutations in the *C. briggsae* AF16 wild-type strain (Fodor *et al.* 1983). Since dauers (but not other stages) survive exposure to the 1% SDS solution (Cassada and Russell 1975; Riddle 1977), in an F_2 clonal screen, we looked for lines that fail to survive in 1% SDS (see materials and methods). From a screen of ~850 mutagenized genomes, we isolated 17 strong Daf-d mutations, of which 14 were kept (Table 2). In addition, the mutation *sy5321* was isolated in a screen for suppressors of *Cb-daf-4(sa973)* (see materials and methods). Since *sy5321* causes a Daf-d phenotype, it was analyzed in parallel. In *C. elegans*, *daf-d* mutants fall into several categories that exhibit distinct phenotypic characteristics. We carried out a set of tests to classify the newly identified *C. briggsae* mutants into analogous classes.

In *C. elegans*, the mutation *daf-13(m66)* causes animals to form morphologically normal dauers that fail to survive exposure to the 1% SDS solution (Riddle *et al.* 1981). We found that *C. briggsae* strains carrying *sy5313*, *sy5416*, or *sy5429* exhibited the same phenotype (Figure 2). Isolation of multiple mutants of this class in *C. briggsae* is not surprising, since the screen for this type of mutants has not been saturated in *C. elegans*. These mutations likely affect specific physiological changes that make dauers more resistant to environmental stresses, but do not affect the mechanism that regulates dauer formation. These mutations were not analyzed further.

In *C. elegans*, mutation of any of a large group of genes (cilium structure genes) causes a defect in the structure of ciliated endings of sensory neurons (Perkins *et al.* 1986; Starich *et al.* 1995). Mutations affecting any of these genes cause a stereotypic set of phenotypes resulting from a general sensory defect. These include defects in dauer formation (Daf-d), osmotic avoidance (Osm), and chemotaxis (Che). Also, several sensory neurons in wild-type animals (both *elegans* and *briggsae*) fill with dyes (FITC, DiI, or DiO) when exposed to them in solution. In cilium structure mutants, the same cells are present, but fail to fill with dye (Dyf, dye-filling defective). In *C. elegans*, it has been reported that a large fraction of randomly isolated Daf-d mutations are of this class (Riddle 1977). We found mutations *sy5311*, *sy5314*, *sy5420*, and *sy5421* were of this class on the basis of the dye-filling phenotype (Table 2) (see materials and methods). Another Daf-d mutation, *sa934*, was isolated in a pilot screen for Dyf mutants.

Many of the remaining mutations likely affect orthologs of *daf-3* (Patterson *et al.* 1997), *daf-5* (Da Graca *et al.* 2004), *daf-12* (Antebi *et al.* 2000), *daf-16* (Lin *et al.* 1997; Ogg *et al.* 1997), or *daf-18* (Ogg and Ruvkun 1998; Gil *et al.* 1999; Mihaylova *et al.* 1999; Rouault *et al.* 1999); wherein the mutations cause the Daf-d

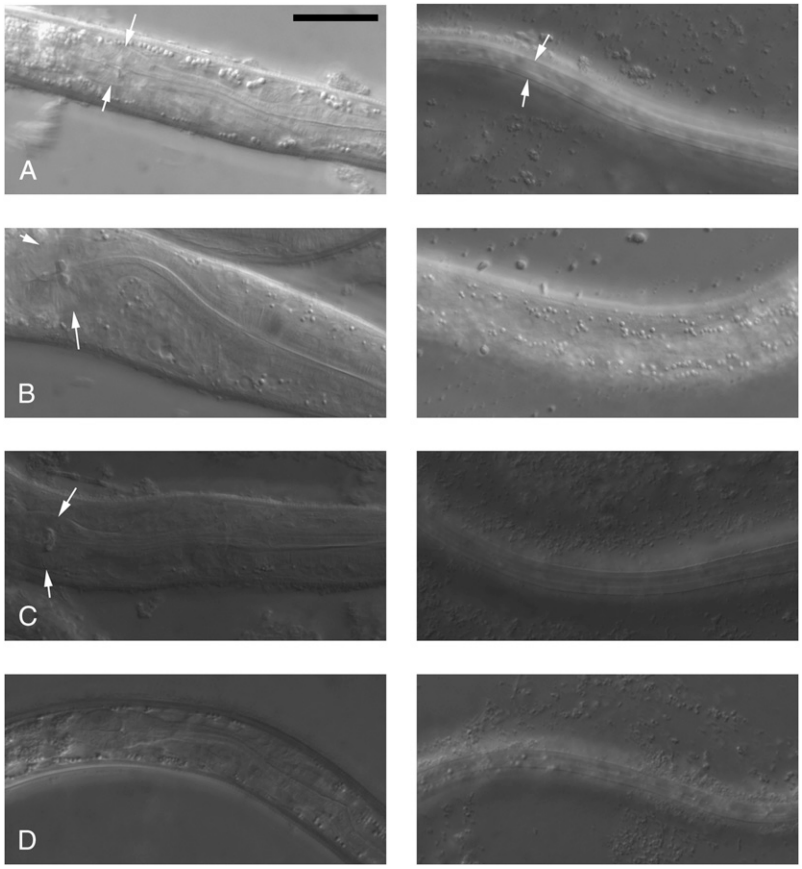

Figure 2.—Partial dauer phenotypes of *sy5313* and *sy5315*. Nomarski images of the pharynx (left) and dauer alae (or absence thereof, right) are shown. Arrows point to the terminal bulb of the pharynx (left) and dauer alae (right). All panels are shown at the same magnification. The scale bar is 20 μm. (A) Wild-type dauer. Pharynx is remodeled (narrower than in non-dauers; compare with panel B), and prominent dauer alae are present (visible as longitudinal stripes). (B) Wild-type non-dauer. Pharynx is wide and dauer alae are absent. (C) *sy5315* partial dauer. Dauer alae are present but the pharynx is not fully remodeled. (D) *sy5313* dauer. Both pharynx and alae are similar to wild-type dauers.

phenotype (Riddle 1977; Riddle *et al.* 1981). Three types of experiments were carried out to help assign mutations to genes (Table 2). First, mutations were tested for linkage to the X chromosome. Chromosome-level synteny appears to be the rule for *elegans–briggsae* comparisons. [Genes on the same chromosome in *C. elegans* are usually found on the same chromosome in *C. briggsae* (Hillier *et al.* 2007).] Thus X-linked mutations could be alleles of *daf-3* and *daf-12* (X-linked in *C. elegans*; Riddle *et al.* 1981). Second, some of the mutations were tested for the ability to suppress *Cb-daf-4(sa973)*. In *C. elegans*, mutations in *daf-3*, *daf-5*, and *daf-12* completely suppress the Daf-c phenotype of *daf-4* mutations (Riddle *et al.* 1981; Vowels and Thomas 1992; Thomas *et al.* 1993). We found that *sy5417* suppressed *Cb-daf-4(sa973)*. Third, strains bearing these mutations were tested for the ability to form partial dauers, animals that exhibit a subset of dauer-like characteristics. In *C. elegans*, all mutations in *daf-16*, *daf-18*, and some mutations in *daf-12* cause animals to form partial dauers (Vowels and Thomas 1992). We found that the *sy5315* bearing strain formed partial dauers in which dauer alae are present but the pharynx is not fully remodeled (Figure 2).

Although X-linked, *sy5315* does not resemble typical *daf-12* alleles, which do not form partial dauers. There is a unique partial dauer-forming *daf-12* allele called *m25*, which is a mutation in the ligand binding domain of *daf-12* (Antebi *et al.* 2000). We sequenced the ligand binding domain of *Cb-daf-12* in a *sy5315* bearing strain (not shown) and found no mutation.

Isolation and characterization of dauer formation constitutive mutations in *C. briggsae*: In *C. elegans*, most Daf-c alleles, including many null alleles, are temperature sensitive for dauer entry because they do not completely eliminate the inherent temperature sensitivity of the pathway (Golden and Riddle 1984a; Malone and Thomas 1994). Such mutations also typically exhibit a temperature-sensitive defect in dauer recovery. Entry into the dauer stage is favored at high temperatures, while exit from the dauer stage is favored at lower temperatures. The original screen for Daf-c mutants in *C. elegans* utilized these characteristics (Riddle 1977), and we carried out an analogous screen in *C. briggsae*. We mutagenized wild-type animals, and dauers were isolated in the F_2 generation at 25° or 28°, temperatures at which wild-type animals do not normally form dauers if not overcrowded and food is present. These dauers were allowed to recover at lower temperatures (20° or 15°) and selfed to establish a line. This screen requires that the mutant we isolate is capable of recovering from the dauer stage when downshifted to the lower temperature. Over 13,000 EMS mutagenized genomes were screened using this procedure and one strong Daf-c mutation, *sa973*, and several weaker mutations were isolated (Table 3; Figure 3). The frequency at which Daf-c mutants were recovered is much lower than the frequency of Daf-c mutations from a

TABLE 3

Daf-c mutants of *C. briggsae*

Mutation	Gene	Class	Linkage	Other
sa973	*Cb-daf-4*		III	
sa974		Weak Daf-c		
sy5331		Weak Daf-c		
sy5367		Weak Daf-c		
sy5445	*Cb-daf-2*		III	Isolated as a *daf-3* revertant

similar screen in *C. elegans* (Riddle 1977). Several factors might contribute to this difference. First, fewer genes in *C. briggsae* may mutate to cause the Daf-c phenotype. Second, a typical Daf-c mutation in *C. briggsae* may fail to recover when downshifted to 20° or 15°. Lastly, since different experimenters in different laboratories carried out these screens, there may be a difference in sensitivities to finding dauers from a large population.

Because a screen for Daf-c mutations by a straightforward F_2 screen failed to produce many mutants, we also carried out a screen for dauer-forming mutants in the *Cb-daf-3* mutant background. *Cb-daf-3(sy5417)* mutants typically do not form any dauers, even at high population density and high temperature. If genetic interactions in *C. briggsae* are similar to those in *C. elegans*, we expect to recover mutations in genes *daf-2* (Kimura *et al.* 1997), *age-1* (Morris *et al.* 1996), *pdk-1* (Paradis *et al.* 1999) (insulin pathway), *daf-11* (guanylyl cyclase) (Birnby *et al.* 2000), and *daf-19* (RFX transcription factor) (Swoboda *et al.* 2000). From these screens, we found one strong mutation, *sy5445* (Table 3; Figure 3). Because the selection was carried out in bulk, the frequency of Daf-c mutations from this screen was not determined (see materials and methods).

***sy5417* has a mutation in *Cb-daf-3* (Smad):** The Daf-d mutation *sy5417* is X-linked and suppresses the Daf-c phenotype of *Cb-daf-4(sa973)*. Since in *C. elegans*, genes *daf-3* and *daf-12* map to the X-chromosome and mutations in these genes suppress *daf-4(−)*, this suggested that *sy5417* is a mutation in an ortholog of one of these genes (Riddle *et al.* 1981). *daf-3* encodes a Smad protein, a cytoplasmic/nuclear transducer of TGF-β and related signals (Patterson *et al.* 1997). We sequenced the *Cb-daf-3* gene from a strain carrying the *sy5417* mutation (see materials and methods) and found that *sy5417* has a missense mutation in the second conserved domain of *Cb-daf-3* (Figure 4).

***sy5445* has a mutation in *Cb-daf-2* (insulin receptor):** Genetic mapping placed the Daf-c mutation *sy5445* on chromosome 3 (see materials and methods). *C. elegans* Daf-c genes *daf-2*, *daf-4*, and *daf-7* map to chromosome 3. However, double mutants *daf-4; daf-3* and *daf-7; daf-3* are non-Daf-c (Riddle *et al.* 1981). Since *sy5445* was isolated as a dauer constitutive mutation in the *Cb-daf-3* mutant background, it was unlikely to be a mutation in *Cb-daf-4* or *Cb-daf-7*. *daf-2* encodes a homolog of an insulin receptor/receptor tyrosine kinase (Kimura *et al.* 1997). We sequenced the *Cb-daf-2* cDNA from the *sy5445* mutant (see materials and methods) and found a missense mutation in the kinase domain of *Cb-daf-2* (Figure 4). The mutation affects a residue conserved in *daf-2* and other insulin receptors, but not conserved in other receptor tyrosine kinases.

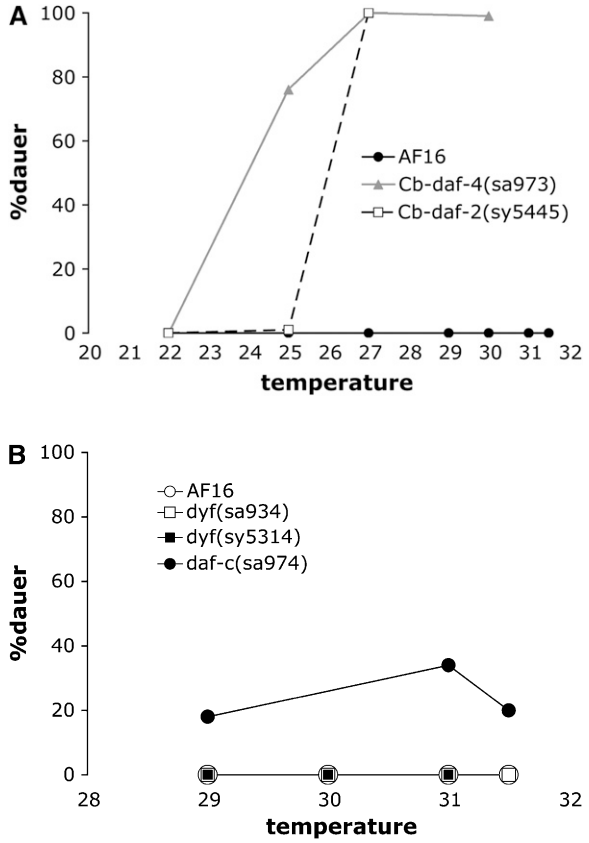

Figure 3.—Dauer formation phenotypes of *C. briggsae* strains. (A) Dauer formation by strong Daf-c mutants. *daf-4(sa973)* strains are Sma (short and small) at 25° and above, but have normal body morphology at lower temperatures. (B) Dauer formation at high temperatures. Unlike in *C. elegans*, *dyf* mutants and weak *daf-c* mutants are not hyperinduced for dauer formation at these temperatures. *Cb-daf-3(sy5417)* and *cby-15(sy5148)* mutants also formed no dauers at 27° or 30° (not shown).

In *C. elegans*, mutations in *daf-2* extend lifespan (Kenyon *et al.* 1993). To test whether the same was true in *C. briggsae*, we measured the survival of *Cb-daf-2(sy5445)* animals at 20°. The lifespan of wild-type AF16 animals (average ± standard deviation) was 19 ± 7.4 and 16 ± 6.5 in two trials. The lifespan of the *Cb-daf-2* mutant was 25 ± 7.9 and 23 ± 7.8 in two trials done in parallel. Thus, as in *C. elegans*, a mutation in *daf-2* increases the lifespan of *C. briggsae* (Figure 5).

***sa973* is a mutation in *Cb-daf-4* (TGF-β receptor type 2):** The Daf-c mutation *sa973* exhibited a temperature-sensitive small-body-size phenotype (Sma). In addition, examination of males at restrictive temperatures revealed

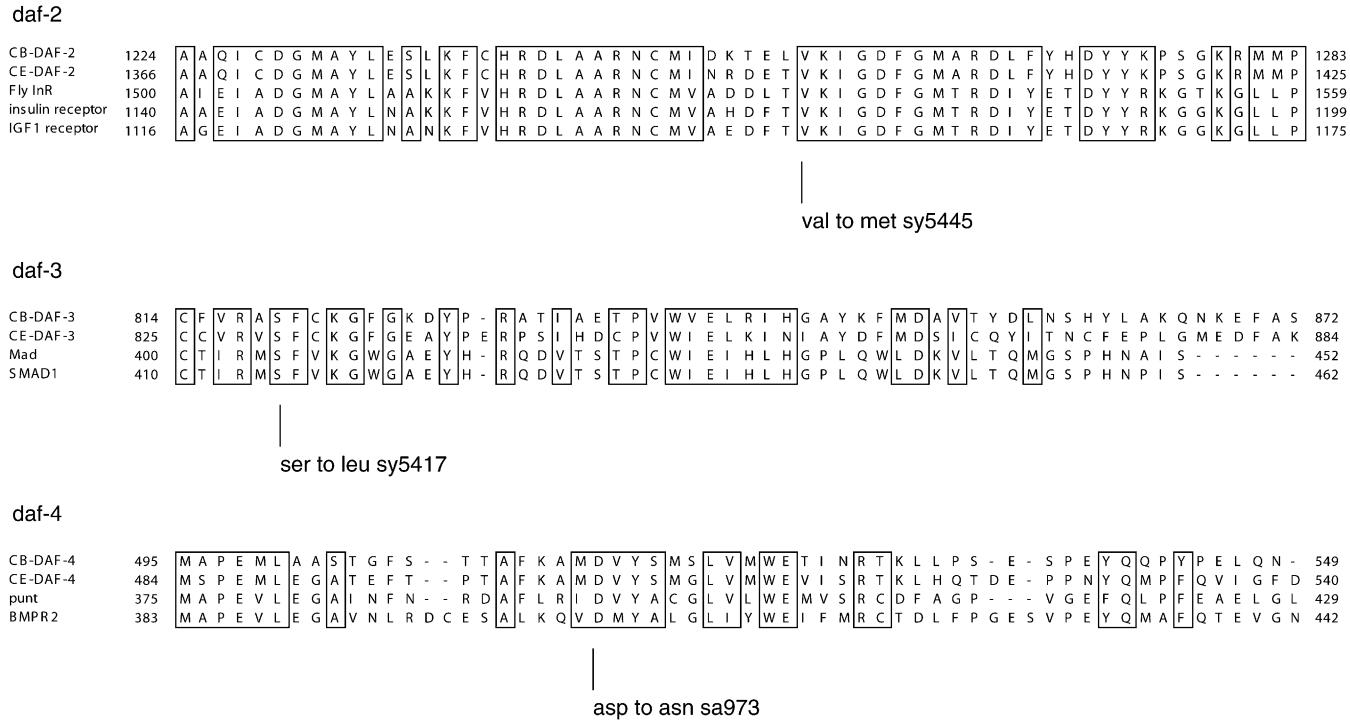

FIGURE 4.—Molecular characterizations of *Cb-daf-2*, *Cb-daf-3*, and *Cb-daf-4* mutations. A sequence alignment of each *C. briggsae* gene with the *C. elegans* ortholog and two or three other arbitrarily selected homologs is shown, and mutated residues are indicated below. The amino acid count is derived from the Wormbase WS150 gene model and may not reflect the actual gene structure.

male tail defects, including crumpled spicules and ray defects (male abnormal; Mab) (Figure 6). In *C. elegans*, this combination of phenotypes (Sma, Mab, and Daf-c) is observed only in *daf-4* mutants (ESTEVEZ *et al.* 1993; SAVAGE *et al.* 1996). *daf-4* encodes a type-2 TGF-β/BMP receptor (ESTEVEZ *et al.* 1993). We transformed the *sa973* mutant strain with a *Ce-daf-4* genomic clone (ESTEVEZ *et al.* 1993) and a transformation marker *Ce-myo-2::gfp* (see MATERIALS AND METHODS) and found that the transgene rescued the Sma phenotype. To determine the molecular nature of the defect, we sequenced the *Cb-daf-4* gene from the *sa973* mutant strain (see MATERIALS AND METHODS) and found a mutation in the kinase domain (Figure 4). A typical *C. elegans daf-4* mutation causes a temperature-sensitive Daf-c phenotype, whereas the Sma phenotype is not temperature sensitive (ESTEVEZ *et al.* 1993). Thus, the fact that the *Cb-daf-4(sa973)* Sma phenotype is temperature sensitive suggests that this missense mutation causes the CB-DAF-4 protein to be temperature sensitive.

Curiously, the *Cb-daf-4(sa973)* strain carrying the *Ce-daf-4(+)* transgene was not obviously rescued for the Daf-c phenotype at 27° (data not shown). To determine whether this was due to mosaicism, in which not all cells in the animal carried the transgene, we integrated the transgene into the genome (Table 4) (see MATERIALS AND METHODS). We found that the integrated lines also did not rescue dauer formation at 27°. Since *Ce-daf-4* rescues the Sma phenotype at 27°, this is not explained solely by the inability of CE-DAF-4 to function at high temperatures. We think a more likely explanation is that the lack of rescue reflects a change in the *cis*-regulatory mechanism of transcriptional regulation and that *Ce-daf-4* is not expressed in the cells where *Cb-daf-4* functions to control dauer formation. A similar type of change in *cis*-regulatory mechanisms has been observed for the *lin-48* gene, required for positioning of the excretory canal in *C. elegans* but not *C. briggsae* (WANG and CHAMBERLIN 2002, 2004).

Absence of high temperature-induced dauer formation in *C. briggsae*: In *C. elegans*, in addition to broad

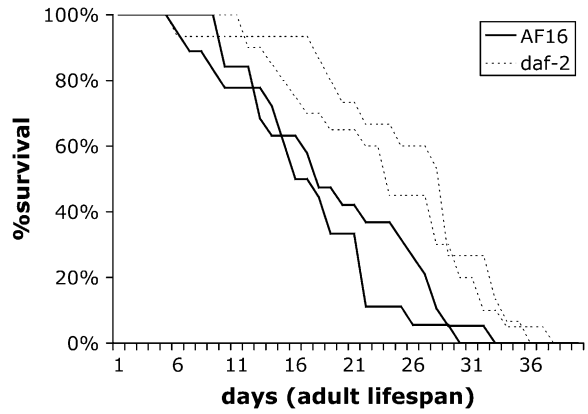

FIGURE 5.—Survival curves of AF16 wild type and *Cb-daf-2(sy5445)* at 20°. Two sets of assays were carried out for each strain. Numbers of animals are 19 and 18 for AF16 data sets and 15 and 20 for *Cb-daf-2(sy5445)* data sets. *P*-values were 0.0131 and 0.0119 (Mann-Whitney test).

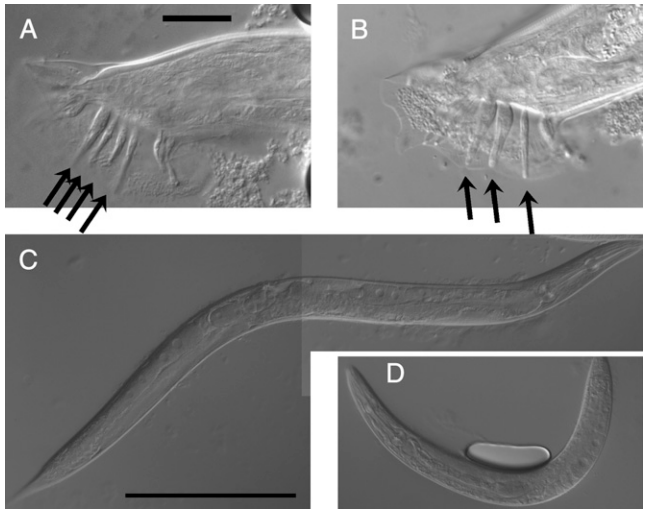

FIGURE 6.—Pleiotropic phenotypes of *Cb-daf-4*. (A) Lateral view of the wild-type male tail. Anterior is to the right. Arrows point to rays 3, 4, 5, and 6 (from right to left). The scale bar is 20 μm. (B) *daf-4(sa973)* male. The thicker ray in the middle is probably a result of ray 4/ray 5 fusion. (C) Wild-type hermaphrodite body shape. (D) The Sma phenotype of the *daf-4(sa973)* hermaphrodite. C and D are shown at the same scale. The scale bar is 250 μm. All animals shown here are progeny of *daf-4*/+ male and hermaphrodite parents grown at 25° on a single plate. Animals in A and C are either wild type or *daf-4*/+ heterozygotes.

temperature sensitivity of the dauer formation process observed over the 15° to 25° temperature range, high temperatures (27° and above) are exceptionally strongly dauer inducing (AILION and THOMAS 2000). Wild-type animals become hypersensitive to dauer pheromone at high temperatures, and also form some dauers in the absence of pheromone. Many mutants that are weakly Daf-c become strongly Daf-c at 27°. In addition, several classes of mutants that are Daf-d at lower temperatures, including *daf-3* and cilium structure mutants, become Daf-c at 27°. It is possible that this response in the wild-type *C. elegans* protects it from temperatures at which they are unable to grow.

To test whether a similar strong induction could be observed in *C. briggsae*, we looked for dauer formation at high temperatures by the wild type (AF16), two cilium structure mutants [*dyf(sa934)* and *dyf(sy5314)*], *Cb-daf-3(sy5417)*, and a weak Daf-c mutant [*daf-c(sa974)*]. Unlike in *C. elegans*, the wild type was not observed to form dauers at temperatures above 27°, nor did they form dauers close to the upper temperature limit of growth and reproduction for *C. briggsae*, 31.5°. In addition, *dyf* mutants and *Cb-daf-3(sy5417)* mutants did not form dauers at high temperatures (Figure 3B). This is in contrast to *C. elegans* where most *dyf* mutations and *daf-3* alleles cause animals to form 100% dauers at 27°. Finally, the weak Daf-c mutant *daf-c(sa974)* formed relatively few dauers at all temperatures we assayed. This again contrasts with *C. elegans*, where all known mutants that form some dauers at 25° form 100% dauers at 27°. These results strongly suggest that the specific high-temperature response we observe in *C. elegans* is not present in *C. briggsae*.

TABLE 4

Integrants of *saEx[myo-2:gfp, Ce-daf-4(+)]*

Integrant	Linkage
syIs802	X
syIs803	II
syIs804	X
syIs807	IV

DISCUSSION

Comparison of the dauer formation pathway in *C. elegans* and *C. briggsae*: Our results demonstrate that the overall pattern of *daf* gene functions and interactions is conserved between *C. elegans* and *C. briggsae*. We isolated mutations in *Cb-daf-2*, *Cb-daf-3*, *Cb-daf-4*, and in genes that affect the formation of sensory cilia. In general, these mutations cause phenotypes very similar to mutations in orthologous *C. elegans* genes: *Cb-daf-3* and cilium structure mutants are Daf-d, whereas *Cb-daf-2* and *Cb-daf-4* mutants are Daf-c. These similarities extend to their respective pleiotropies. The *Cb-daf-2* mutant was long-lived, the *Cb-daf-4* mutant was small and exhibited male tail defects, and cilium structure mutants were Dyf. Furthermore, the pattern of interactions among these genes is conserved. In particular, *Cb-daf-3(sy5417)* suppresses *Cb-daf-4(sa973)*, and *Cb-daf-2(sy5445)* is epistatic to *Cb-daf-3(sy5417)*. Thus, the pathways regulating dauer formation are largely conserved between *C. elegans* and *C. briggsae*.

In contrast to the overall conservation of pathways, *C. briggsae* and *C. elegans* differ in their responses to high temperatures. Temperatures above 26° are strongly dauer promoting for *C. elegans* (AILION and THOMAS 2000). Our examination of *C. briggsae* wild-type and mutant (both Daf-d and Daf-c) animals at 27° and 31.5° strongly argues against the presence of a similar response in *C. briggsae*. Related Caenorhabditis species [*e.g.*, *C. remanei*, *C. sp.* PS1010, *C. brenneri* (SUDHAUS and KIONTKE 2007)] are able to grow at higher temperatures than *C. elegans* (our data not shown), suggesting that this may be a recently acquired behavioral response in *C. elegans*. Further molecular analysis of the dauer formation response in *C. briggsae* and *C. elegans* potentially can shed light on mechanisms by which neuronally controlled behaviors change during evolution.

Forward genetic analysis in *C. briggsae*: Increasingly, *C. briggsae* is becoming an important model organism for comparative studies. Aspects of *C. briggsae* biology that are under investigation include positioning of the excretory duct cell (WANG and CHAMBERLIN 2002, 2004), functions of Notch receptors *glp-1* and *lin-12*, (RUDEL and KIMBLE 2001, 2002), male tail development (BAIRD

et al. 2005), vulval development (Delattre and Felix 2001), and molecular evolution of gene families (*e.g.*, Thomas *et al.* 2005). One particularly fruitful line of investigation examined the mechanism of sex determination. Molecular and phylogenetic analyses indicate that hermaphroditism in *C. elegans* and *C. briggsae* evolved independently from male/female ancestors (Cho *et al.* 2004; Kiontke *et al.* 2004). Molecular analyses of the sex-determination gene *fog-2* and functional analyses of genes including *gld-1*, *fem-2*, and *fem-3* revealed significant differences in mechanisms by which hermaphrodites are produced in *C. elegans* and *C. briggsae*, consistent with convergent, independent evolution of hermaphroditism (Hill *et al.* 2006; Nayak *et al.* 2005). Hill *et al.* (2006) also highlighted an important technical point: although progress can be made with the use of RNAi, because RNAi is often variable and does not fully eliminate gene function, chromosomal null and reduction-of-function mutations are needed to rigorously compare functions of genes in the two species.

Ours is one of the first systematic efforts to generate a large number of mutations affecting a single process in *C. briggsae*, and our results demonstrate that forward genetic analysis in *C. briggsae* can be rapid. In general, a mutagenesis and a screen are followed by basic characterizations of isolated alleles. Pleiotropic phenotypes and mapping data provide clues to the molecular identity on the basis of homology to *C. elegans*, and the identity can be ascertained rapidly by transformation rescue or by direct sequencing of the gene. Once mutations are identified in a few key genes, additional reagents may be generated easily by using preexisting alleles, as demonstrated by our isolation of a *Cb-daf-2* allele from a reversion screen of *Cb-daf-3*. Null alleles, which are necessary for quantitative comparisons of gene functions, can be generated from non-null alleles of the same gene by noncomplementation screens.

A similar conclusion is also true for a nematode more distantly related to *C. elegans*, *Pristionchus pacificus*, where a number of mutations from forward genetic analyses have been cloned molecularly (Sommer 2006). *C. briggsae* is much more closely related to *C. elegans* and thus shows a greater degree of phenotypic similarity and synteny (Stein *et al.* 2003). Moreover, the availability of the genome sequence, a genetic map with a large number of visible mutations, and a collection of SNP (single nucleotide polymorphism) markers raise the possibility of easily cloning genes *de novo* in *C. briggsae* without relying on homology to *C. elegans* (Gupta *et al.* 2007). The closer relationship of *C. briggsae* to *C. elegans* means that different types of evolutionary changes will be studied by comparisons of these two species than in comparisons between *C. elegans* and *P. pacificus*. Neuronally controlled traits, such as dauer formation, may be more variable between Caenorhabiditis species than morphological traits like vulval development, which is being investigated in *C. elegans* and *P. pacificus* (Sommer 2006).

Genetic tools for study of *C. briggsae*: Our work on dauer formation generated useful reagents, which will facilitate analysis of gene functions in *C. briggsae* using mutants. For example, we integrated transgenic arrays containing *myo-2::gfp* and *Ce-daf-4(+)* on chromosomes 2, 4, and X. Since *myo-2::gfp* is a bright fluorescent reporter expressed in all stages, these integrants can be used as dominant markers for these chromosomes in mapping experiments and strain constructions. The X-linked *myo-2::gfp* integrant *syIs802* has also proved to be useful because *syIs802/+* heterozygotes produce high frequency of XO male progeny, probably by interfering with meiotic pairing (Hill *et al.* 2006). Finally, molecularly characterized mutations with easily scored phenotypes contribute to increasing the density of the *C. briggsae* genetic map.

We thank R. Johnsen and D. Baillie for *C. briggsae* mutants and mapping data. We thank S. Gharib and B. P. Gupta for technical assistance and useful reagents. We thank E. Hallem, X. Wang, and J. Srinivasan for comments on the manuscript. T.I. was supported by fellowship DRG-1646 from the Damon Runyon Cancer Research Foundation. This work was supported by HHMI, where P.W.S. is an investigator. Some nematode strains used in this work were provided by the Caenorhabditis Genetics Center, which is funded by the National Institutes of Health National Center for Research Resources (NCRR).

LITERATURE CITED

Ailion, M., and J. H. Thomas, 2000 Dauer formation induced by high temperatures in *Caenorhabditis elegans*. Genetics **156:** 1047–1067.

Antebi, A., W. H. Yeh, D. Tait, E. M. Hedgecock and D. L. Riddle, 2000 *daf-12* encodes a nuclear receptor that regulates the dauer diapause and developmental age in *C. elegans*. Genes Dev. **14:** 1512–1527.

Baird, S. E., C. R. Davidson and J. C. Bohrer, 2005 The genetics of ray pattern variation in *Caenorhabditis briggsae*. BMC Evol. Biol. **5:** 3.

Birnby, D. A., E. M. Link, J. J. Vowels, H. Tian, P. L. Colacurcio *et al.*, 2000 A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in *Caenorhabditis elegans*. Genetics **155:** 85–104.

Brenner, S., 1974 The genetics of *Caenorhabditis elegans*. Genetics **77:** 71–94.

Butcher, R. A., M. Fujita, F. C. Schroeder and J. Clardy, 2007 Small-molecule pheromones that control dauer development in *Caenorhabditis elegans*. Nat. Chem. Biol. **3:** 420–422.

Cassada, R. C., and R. L. Russell, 1975 The dauerlarva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. **46:** 326–342.

Cho, S., S. W. Jin, A. Cohen and R. E. Ellis, 2004 A phylogeny of *Caenorhabditis* reveals frequent loss of introns during nematode evolution. Genome Res. **14:** 1207–1220.

Cutter, A. D., M. A. Felix, A. Barriere and D. Charlesworth, 2006 Patterns of nucleotide polymorphism distinguish temperate and tropical wild isolates of *Caenorhabditis briggsae*. Genetics **173:** 2021–2031.

da Graca, L. S., K. K. Zimmerman, M. C. Mitchell, M. Kozhan-Gorodetska, K. Sekiewicz *et al.*, 2004 DAF-5 is a Ski oncoprotein homolog that functions in a neuronal TGF beta pathway to regulate *C. elegans* dauer development. Development **131:** 435–446.

Delattre, M., and M. A. Felix, 2001 Polymorphism and evolution of vulval precursor cell lineages within two nematode genera, Caenorhabditis and Oscheius. Curr. Biol. **11:** 631–643.

Estevez, M., L. Attisano, J. L. Wrana, P. S. Albert, J. Massague *et al.*, 1993 The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. Nature **365:** 644–649.

Fodor, A., D. L. Riddle, F. K. Nelson and J. W. Golden, 1983 Comparison of a new wild type *Caenorhabditis briggsae* with

laboratory strains of *C. briggsae* and *C. elegans*. Nematologica **29:** 203–217.

Gil, E. B., E. Malone Link, L. X. Liu, C. D. Johnson and J. A. Lees, 1999 Regulation of the insulin-like developmental pathway of *Caenorhabditis elegans* by a homolog of the PTEN tumor suppressor gene. Proc. Natl. Acad. Sci. USA **96:** 2925–2930.

Golden, J. W., and D. L. Riddle, 1982 A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science **218:** 578–580.

Golden, J. W., and D. L. Riddle, 1984a A pheromone-induced developmental switch in *Caenorhabditis elegans*: Temperature-sensitive mutants reveal a wild-type temperature-dependent process. Proc. Natl. Acad. Sci. USA **81:** 819–823.

Golden, J. W., and D. L. Riddle, 1984b The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. Dev. Biol. **102:** 368–378.

Gupta, B. P., R. Johnsen and N. Chen, 2007 Genomics and biology of the nematode *Caenorhabditis briggsae* (May 3, 2007), in *WormBook*, edited by The *C. elegans* Research Community (http://www.wormbook.org).

Hill, R. C., C. E. de Carvalho, J. Salogiannis, B. Schlager, D. Pilgrim *et al.*, 2006 Genetic flexibility in the convergent evolution of hermaphroditism in Caenorhabditis nematodes. Dev. Cell **10:** 531–538.

Hillier, L. W., R. D. Miller, S. E. Baird, A. Chinwalla, L. A. Fulton *et al.*, 2007 Comparison of *C. elegans* and *C. briggsae* genome sequences reveals extensive conservation of chromosome organization and synteny. PLoS Biol. **5:** e167.

Hodgkin, J., 1995 *Caenorhabditis elegans*. Trends Genet. Genetic Nomenclature Guide, 24–25.

Horvitz, H. R., S. Brenner, J. Hodgkin and R. K. Herman, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

Jeong, P.-Y., M. Jung, Y.-H. Yim, H. Kim, M. Park *et al.*, 2005 Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. Nature **433:** 541–545.

Kennedy, B. P., E. J. Aamodt, F. L. Allen, M. A. Chung, M. F. Heschl *et al.*, 1993 The gut esterase gene (*ges-1*) from the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae*. J. Mol. Biol. **229:** 890–908.

Kenyon, C., J. Chang, E. Gensch, A. Rudner and R. Tabtiang, 1993 A *C. elegans* mutant that lives twice as long as wild type. Nature **366:** 461–464.

Kimura, K. D., H. A. Tissenbaum, Y. Liu and G. Ruvkun, 1997 *daf-2*, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science **277:** 942–946.

Kiontke, K., N. P. Gavin, Y. Raynes, C. Roehrig, F. Piano *et al.*, 2004 Caenorhabditis phylogeny predicts convergence of hermaphroditism and extensive intron loss. Proc. Natl. Acad. Sci. USA **101:** 9003–9008.

Kirouac, M., and P. W. Sternberg, 2003 cis-Regulatory control of three cell fate-specific genes in vulval organogenesis of *Caenorhabditis elegans* and *C. briggsae*. Dev. Biol. **257:** 85–103.

Lin, K., J. B. Dorman, A. Rodan and C. Kenyon, 1997 *daf-16*: An HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. Science **278:** 1319–1322.

Malone, E. A., and J. H. Thomas, 1994 A screen for nonconditional dauer-constitutive mutations in *Caenorhabditis elegans*. Genetics **136:** 879–886.

Mihaylova, V. T., C. Z. Borland, L. Manjarrez, M. J. Stern and H. Sun, 1999 The PTEN tumor suppressor homolog in *Caenorhabditis elegans* regulates longevity and dauer formation in an insulin receptor-like signaling pathway. Proc. Natl. Acad. Sci. USA **96:** 7427–7432.

Morris, J. Z., H. A. Tissenbaum and G. Ruvkun, 1996 A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature **382:** 536–539.

Nayak, S., J. Goree and T. Schedl, 2005 *fog-2* and the evolution of self-fertile hermaphroditism in *Caenorhabditis*. PLoS Biol. **3:** e6.

Ogg, S., and G. Ruvkun, 1998 The *C. elegans* PTEN homolog, DAF-18, acts in the insulin receptor-like metabolic signaling pathway. Mol. Cell **2:** 887–893.

Ogg, S., S. Paradis, S. Gottlieb, G. I. Patterson, L. Lee *et al.*, 1997 The Fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature **389:** 994–999.

Paradis, S., M. Ailion, A. Toker, J. H. Thomas and G. Ruvkun, 1999 A PDK1 homolog is necessary and sufficient to transduce AGE-1 PI3 kinase signals that regulate diapause in *Caenorhabditis elegans*. Genes Dev. **13:** 1438–1452.

Patterson, G. I., A. Koweek, A. Wong, Y. Liu and G. Ruvkun, 1997 The DAF-3 Smad protein antagonizes TGF-beta-related receptor signaling in the *Caenorhabditis elegans* dauer pathway. Genes Dev. **11:** 2679–2690.

Perkins, L. A., E. M. Hedgecock, J. N. Thomson and J. G. Culotti, 1986 Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. **117:** 456–487.

Prasad, B. C., B. Ye, R. Zackhary, K. Schrader, G. Seydoux *et al.*, 1998 *unc-3*, a gene required for axonal guidance in *Caenorhabditis elegans*, encodes a member of the O/E family of transcription factors. Development **125:** 1561–1568.

Riddle, D. L., 1977 A genetic pathway for dauer larva formation in *Caenorhabditis elegans*. Stadler Genet. Symp. 9: 101–120.

Riddle, D. L., M. M. Swanson and P. S. Albert, 1981 Interacting genes in nematode dauer larva formation. Nature **290:** 668–671.

Rouault, J. P., P. E. Kuwabara, O. M. Sinilnikova, L. Duret, D. Thierry-Mieg *et al.*, 1999 Regulation of dauer larva development in *Caenorhabditis elegans* by *daf-18*, a homologue of the tumour suppressor PTEN. Curr. Biol. **9:** 329–332.

Rudel, D., and J. Kimble, 2001 Conservation of *glp-1* regulation and function in nematodes. Genetics **157:** 639–654.

Rudel, D., and J. Kimble, 2002 Evolution of discrete Notch-like receptors from a distant gene duplication in *Caenorhabditis*. Evol. Dev. **4:** 319–333.

Savage, C., P. Das, A. L. Finelli, S. R. Townsend, C. Y. Sun *et al.*, 1996 *Caenorhabditis elegans* genes *sma-2, sma-3,* and *sma-4* define a conserved family of transforming growth factor beta pathway components. Proc. Natl. Acad. Sci. USA **93:** 790–794.

Sommer, R. J., 2006 *Pristionchus pacificus* (August 14, 2006), *WormBook*, edited by The *C. elegans* Research Community (http://www.wormbook.org).

Starich, T. A., R. K. Herman, C. K. Kari, W. H. Yeh, W. S. Schackwitz *et al.*, 1995 Mutations affecting the chemosensory neurons of *Caenorhabditis elegans*. Genetics **139:** 171–188.

Stein, L. D., Z. Bao, D. Blasiar, T. Blumenthal, M. R. Brent *et al.*, 2003 The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. PLoS Biol. **1:** e45.

Sudhaus, W., and K. Kiontke, 2007 Comparison of the cryptic nematode species *Caenorhabditis brenneri* sp. n. and *C. remanei* (Nematoda: Rhabditidae) with the stem species pattern of the *Caenorhabditis Elegans* group. Zootaxa **1456:** 45–62.

Sulston, J. E., and H. R. Horvitz, 1977 Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

Swoboda, P., H. T. Adler and J. H. Thomas, 2000 The RFX-type transcription factor DAF-19 regulates sensory neuron cilium formation in *C. elegans*. Mol. Cell **5:** 411–421.

Thomas, J. H., 1993 Chemosensory regulation of development in *C. elegans*. Bioessays **15:** 791–797.

Thomas, J. H., D. A. Birnby and J. J. Vowels, 1993 Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics **134:** 1105–1117.

Thomas, J. H., J. L. Kelley, H. M. Robertson, K. Ly and W. J. Swanson, 2005 Adaptive evolution in the SRZ chemoreceptor families of *Caenorhabditis elegans* and *Caenorhabditis briggsae*. Proc. Natl. Acad. Sci. USA **102:** 4476–4481.

Vowels, J. J., and J. H. Thomas, 1992 Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics **130:** 105–123.

Wang, X., and H. M. Chamberlin, 2002 Multiple regulatory changes contribute to the evolution of the *Caenorhabditis lin-48* ovo gene. Genes Dev. **16:** 2345–2349.

Wang, X., and H. M. Chamberlin, 2004 Evolutionary innovation of the excretory system in *Caenorhabditis elegans*. Nat. Genet. **36:** 231–232.

Way, J. C., and M. Chalfie, 1989 The *mec-3* gene of *Caenorhabditis elegans* requires its own product for maintained expression and is expressed in three neuronal cell types. Genes Dev. **3:** 1823–1833.

Communicating editor: D.I. Greenstein